

CLAIMS

1. A method of diagnosing a neoplastic tissue in a human comprising:
detecting amplification of human MDM2 gene or elevated expression of a human MDM2 gene product in a tissue or body fluid isolated from a human, wherein amplification of the human MDM2 gene or elevated expression of human MDM2 gene product provides a diagnosis of neoplasia or the potential for neoplastic development.
2. The method of claim 1 wherein gene amplification is detected.
3. The method of claim 1 wherein elevated expression of a gene product is detected, said gene product being mRNA.
4. The method of claim 1 wherein elevated expression of a gene product is detected, said gene product being human MDM2 protein.
5. The method of claim 3 wherein said mRNA is detected by Northern blot analysis by hybridizing mRNA from said tissue to a human MDM2 nucleotide probe.
6. The method of claim 5 wherein the human MDM2 nucleotide probe comprises nucleotides 1-2372 of human MDM2, as shown in Figure 1, or fragments thereof consisting of at least 14 contiguous nucleotides.
7. The method of claim 4 wherein human MDM2 protein is detected by Western Blot analysis by reacting human MDM2 proteins with antibodies which are immunospecific for MDM2 protein.
8. The method of claim 2 wherein the gene amplification is detected using polymerase chain reaction.
9. The method of claim 2 wherein amplification of the human MDM2 gene is detected by Southern blot analysis wherein the human MDM2 gene is hybridized with a nucleotide probe which is complementary to hMDM2 DNA.
10. The method of claim 2 wherein gene amplification is determined by comparing the copy number of hMDM2 in the tissue to the copy number of hMDM2 in a normal tissue of the human.

09066724-100101

11. The method of claim 3 wherein elevated expression of mRNA is determined by comparing the amount of hMDM2 mRNA in the tissue to the amount of hMDM2 mRNA in a normal tissue of the human.

12. The method of claim 4 wherein elevated expression of hMDM2 protein is determined by comparing the amount of hMDM2 protein in the tissue to the amount of hMDM2 protein in a normal tissue of the human.

13. The method of claim 2 wherein gene amplification is detected when at least 3-fold more hMDM-2 DNA is observed in the tissue relative to a control sample comprising a normal tissue.

14. The method of claim 3 wherein elevated expression is detected when at least 3-fold more hMDM-2 mRNA is observed in the tissue relative to a control sample comprising a normal tissue.

15. The method of claim 4 wherein elevated expression is detected when at least 3-fold more hMDM2 protein is observed in the tissue relative to a control sample comprising a normal tissue.

16. The method of claim 1 wherein the neoplasia is a sarcoma.

17. The method of claim 16 wherein the sarcoma is a liposarcoma, malignant fibrous histiocyoma, or osteosarcoma.

18. A cDNA molecule comprising nucleotides 1 to 2372, as shown in Figure 1, or fragments thereof, consisting of at least 14 contiguous nucleotides.

19. The cDNA molecule of claim 18 comprising the coding sequence of human MDM2.

20. Human MDM2 protein substantially free of other human proteins.

21. A preparation of antibodies specifically immunoreactive with human MDM2 protein.

22. The preparation of claim 21 wherein the antibodies are monoclonal antibodies.

23. A nucleotide probe comprising a sequence of at least 10 nucleotides which are complementary to nucleotides 1-2372 of human MDM2 gene, as shown in Figure 1.

24. A kit for detecting the amplification of a human MDM2 gene in a human tissue or body fluid sample comprising: a nucleic acid probe capable of hybridizing to said human MDM2 gene under conditions of high stringency, and instructions for determining said amplification.

25. A kit for detecting elevated expression of a human MDM2 mRNA in a human tissue or body fluid sample comprising: a nucleic acid probe capable of hybridizing to said mRNA, and written instructions for determining elevated expression of mRNA.

26. A kit for detecting elevated expression of a human MDM2 protein in a human tissue or body fluid sample comprising MDM2 protein-specific antibodies and written instructions for determining elevated expression of human MDM2 protein.

27. A method of treating a neoplastic cell or a cell having neoplastic potential, comprising:

administering to a cell a therapeutically effective amount of an inhibitory compound which interferes with the expression of human MDM2 gene.

28. The method of claim 27 wherein expression of the human MDM2 gene is inhibited by administering antisense oligonucleotides.

29. The method of claim 27 wherein expression of the human MDM2 gene is inhibited by administering triple-strand forming oligonucleotides which interact with DNA.

30. A method for identifying compounds which interfere with the binding of human MDM-2 to human p53, comprising:

binding a predetermined quantity of a first human protein which is detectably labelled to a second human protein;

adding a compound to be tested for its capacity to inhibit binding of said first and second proteins to each other;

determining the quantity of the first human protein which is displaced from or prevented from binding to the second human protein;

0906724.100101

wherein the first human protein is MDM-2 and the second human protein is p53 or the first human protein is p53 and the second human protein is MDM-2.

31. The method of claim 30 wherein one of said two human proteins is fixed to a solid support.

32. The method of claim 30 wherein an antibody specifically immunoreactive with said second human protein is used to separate first human protein bound from unbound first human protein.

33. A method for inhibiting the growth of tumor cells which contain a human MDM2 gene amplification, comprising:

administering a polypeptide to tumor cells which contain a human MDM2 gene amplification, said polypeptide consisting essentially of a portion of p53, said portion comprising amino acids 13-41 of p53, said polypeptide being capable of binding to human MDM2.

34. The method of claim 33 wherein said polypeptide comprises amino acids 1-41 of p53.

35. The method of claim 33 wherein said polypeptide comprises amino acids 13-57 of p53.

36. The method of claim 33 wherein said polypeptide comprises amino acids 1-50 of p53.

37. A method for inhibiting the growth of tumor cells which contain a human MDM2 gene amplification, comprising:

administering to tumor cells which contain a human MDM2 gene amplification a DNA molecule which expresses a polypeptide consisting essentially of a portion of p53, said portion comprising amino acids 13-41 of p53, said polypeptide being capable of binding to human MDM2.

38. The method of claim 37 wherein said polypeptide comprises amino acids 1-41 of p53.

096674-100101
T.D.D.T. 4229650

39. The method of claim 37 wherein said polypeptide comprises amino acids 13-57 of p53.
40. The method of claim 37 wherein said polypeptide comprises amino acids 1-50 of p53.
41. A polypeptide consisting essentially of a portion of p53, said portion comprising amino acids 13-41 of p53, said polypeptide capable of binding to human MDM2.
42. The polypeptide of claim 41 which comprises amino acids 1-41 of p53.
43. The polypeptide of claim 41 which comprises amino acids 13-57 of p53.
44. The polypeptide of claim 41 which comprises amino acids 1-50 of p53.
45. The preparation of claim 21 wherein the antibodies do not bind to other human proteins.
46. The preparation of claim 21 wherein the antibodies do not bind to human proteins of M_r 75-85K, 105-120K, and 170-200K.
47. The preparation of claim 21 wherein the antibodies bind to the epitope bound by antibodies secreted by hybridoma IF2 (ATCC HB 11290).
48. The preparation of claim 21 wherein the antibodies bind to the epitope bound by antibodies secreted by hybridoma ED9 (ATCC HB 11291).
49. The method of claim 7 wherein the antibodies bind to the epitope on hMDM2 bound by antibodies secreted by hybridoma IF2 (ATCC HB 11290).
50. The method of claim 4 wherein human MDM2 protein is detected by immunohistochemistry.
51. The method of claim 50 wherein antibodies are employed in the immunohistochemistry which bind to an epitope on hMDM2 bound by the antibodies secreted by ED9 (ATCC HB 11291).
52. The method of claim 50 wherein antibodies are employed in the immunohistochemistry which bind to an epitope on hMDM2 bound by the antibodies secreted by IF2 (ATCC HB 11290).

0966724-100101

53. The method of claim 4 wherein human MDM2 protein is detected by immunoprecipitation.

54. A hybridoma cell having the identifying characteristics of ED9 (ATCC HB 11291).

55. A hybridoma cell having the identifying characteristics of IF2 (ATCC HB 11290).

039674 100101